Review – hypothesis article

The control of partitioning between protein and fat during human starvation: its internal determinants and biological significance

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Human subjects vary in the extent to which their body's protein and fat compartments are mobilized for fuel during starvation. Although an inverse association between the initial adiposity and the contribution of protein as fuel during starvation has been known for nearly a century, interest in the quantitative importance and functional significance of the initial percentage fat as a determinant of biological variation in energy-partitioning between protein and fat (and hence in determining the partitioning characteristic of the individual) is relatively recent. The present paper addresses these issues by revisiting the classic Minnesota experiment of semi-starvation and refeeding from a standpoint of system physiology. In a quantitative analysis of the relationship between the initial body composition (ratio FAT_0 : fat-free mass $(FFM)_0$) and the composition of weight loss (ratio $\Delta FAT : \Delta FFM$) in the thirty-two men in the Minnesota study, the arguments are put forward that the fraction of FFM lost when the fat stores reach total depletion is independent of the initial percentage fat, and that this fraction represents the 'dispensible' component of the protein compartment that is compatible with life (i.e. the protein energy-reserve, r_p). The concepts are developed that (1) the initial percentage body fat (which reflects the initial ratio $FAT_0 : FFM_0$) provides a 'memory of partitioning' which dictates the control of partitioning between protein and fat in such a way that both the protein energy-reserve (r_p) and the fat energy-reserve (r_f) reach complete depletion simultaneously, a strategy that would ensure maximum length of survival during long-term food scarcity, and that (2) variability in the relative sizes of these two energy reserves (i.e. in $r_f : r_p$) could, in addition to the initial percentage fat, also contribute to human variability in energy-partitioning. The basic assumptions underlying this re-analysis of the Minnesota data, and the concepts that are derived from it, have been integrated in the simple mathematical model for predicting the partitioning characteristic of the individual. This model is used to explain how variability in the fraction of the protein compartment that could function as an energy reserve (r_p) can be as important as the initial percentage fat in determining inter-individual variability in protein-sparing during the early phase of starvation, in fuel partitioning during prolonged starvation, or in the maximum percentage weight loss during starvation. The elucidation of factors underlying variability in the size of the protein energy-reserve may have important implications for our understanding of the pathophysiology of starvation and age-associated susceptibility to muscle wasting, and in the clinical management of cachexia and obesity.

Cachexia: Obesity: Malnutrition: Energy metabolism: Body composition

Life is sustained by chemical energy derived from the combustion of substrates whose continuous supply is ensured through the mobilization of the body's fuel reserves whenever the external fuels supply from foods cannot meet the demands. Whereas on a day-to-day basis a small amplitude of negative energy balance can be buffered by the glycogen stores, the fuel needs during more prolonged food deprivation are derived from the catabolism of the body's protein and fat. The relative proportion of energy withdrawn from these two compartments, referred to as energy-partitioning, and

Abbreviations: FFM, fat-free mass; Pc, partitioning characteristic; P_{ratio}, fraction of energy mobilized as protein during total food deprivation; r_f, fat-reserve fraction; r_p, protein-reserve fraction.

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numerically defined as the fraction of energy mobilized as protein during food deprivation (P_{ratio}), is well known to be a variable. It is dependent on external factors (e.g. the degree of starvation, composition of the restricted diet, intensity of physical activity, resistance training); but also implicated in the variability in P_{ratio} are internal factors which have been postulated to confer biological variability, and which hence determine the partitioning characteristic (Pc) of the individual. According to Payne & Dugdale (1977), the existence of such an internal (autoregulatory) control of energy-partitioning between protein and fat, which they stipulate is highly variable between individuals, but constant within the same individual, is central to the regulation of body weight and body composition. A conceptual representation of Pc during starvation, and its distinction from P_{ratio}, is presented in Fig. 1. The present paper addresses the subject of partitioning between protein and fat strictly in the context of body composition regulation in response to starvation in the adult human subject, and from a standpoint of normal physiology, such that concepts and their integrations are based primarily on the response of healthy individuals to starvation.

Testing the notion of inter- and intra-individual variability in P_{ratio}

By re-analysing data on prolonged fasting (total starvation) conducted earlier this century in normal-weight men, Henry *et al*. (1988) were able to demonstrate that the partitioning between protein and fat is indeed an individual characteristic on the basis that the P_{ratio} remained relatively constant within the individual during the course of the fast, but varied considerably among individuals. For the calculation of P_{ratio}, advantage was taken of the fact that during fasting, since no food was eaten and physical activity was minimal, the amount of protein mobilized could be calculated from 24 h urinary N losses, and the total 24 h energy loss taken to be equal to the BMR. The data in Fig. 2 are from the fasting subject studied by Benedict (1915), and they illustrate the marked contrast between the constancy of the P_{ratio} and the continuous variation of both N loss and BMR which have decreased by more than 25 % at the end of the fast. The P_{ratio} value of 0·187 indicates that on the third day and thereafter, approximately 19 % of the energy loss from this subject's body tissues was derived from protein catabolism. Using the same approach for calculating the P_{ratio} in nine other healthy normal-weight individuals subjected to prolonged fasting, Henry *et al*. (1988) found that the results paralleled those obtained from Benedict's (1915) data in that the P_{ratio}, after a few days following the onset of fasting, remained relatively constant during the course of the fast within a given individual, but was variable from one individual to another. These studies therefore clearly demonstrate inter-individual variability, but intra-individual constancy, in the pattern of lean and fat tissue mobilization, albeit during prolonged fasting in normal-weight men,

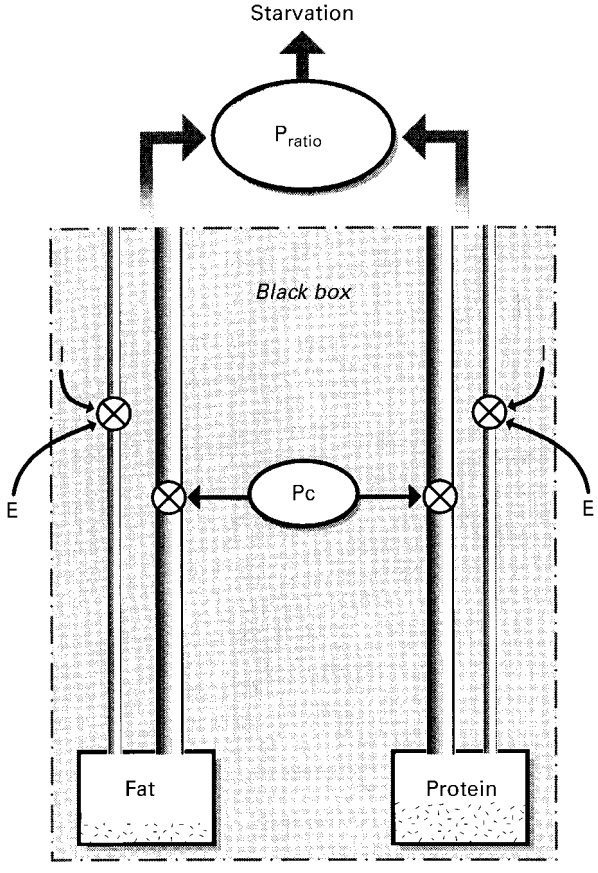

Fig. 1. Inside the black box of body energy-partitioning during starvation. Schematic diagram encapsulating the concept of an individual's partitioning characteristic (Pc) between the two main energy compartments of the body (protein and fat), and the distinction between Pc and the fraction of energy mobilized as protein (P_{ratio}) during the course of starvation. In a given adult individual, the Pc is assumed to be a constant, independent of time, whereas the P_{ratio} reflects the integrated outcome of the Pc and other internal perturbations (e.g. protein sparing during early starvation) or external perturbations (e.g. extremes of physical activity, dietary composition); the curved arrows from within or from outside the black box represent the internal (I) and external (E) perturbations respectively. The two body compartments (protein and fat) are strictly energetic compartments, and refer to the protein-energy compartment (including protein in adipose tissues) and the fat-energy compartment (including lipids in lean tissues such as intramuscular lipids, and membrane lipids). Each of these two compartments is divided into an indispensible component (represented by the filled fraction of the compartment box) and a dispensible component which is conceptualized as the fraction of the compartment that can be lost without irreversible or lethal consequences. Note: (a) In their paper, Payne & Dugdale (1977) refer to the Pc of the individual as P_{ratio}. This has led to a debate regarding the validity of assuming intra-individual constancy of P_{ratio} since the P_{ratio} (defined as the fraction of energy mobilized as protein) of a given individual can clearly vary during starvation (Dulloo, 1998; Henry *et al.* 1998; Millward & Wijesinghe, 1998). However, Payne & Dugdale (1977) were describing a computer simulation of energy-partitioning (presumably in the absence of external or internal perturbations on the control of partitioning) and in that context their reference to P_{ratio} could be equated with Pc depicted in this diagram. (b) In the present state of knowledge, however, it is not known whether the Pc of the individual is entirely genetically determined, whether it is entirely the result of previous exposure to external factors, or whether the phenotypic expression of the Pc is a combination of genetic predisposition and previous exposure to external factors. (c) The representation of the body compartments in this diagram (strictly in energetic terms as mentioned earlier) also differs from the Payne–Dugdale model for changes in energy balance and body weight, in which the body is divided into four 'mass' rather than 'energy' compartments as follows: tissue fat store (e.g. adipose storage), structural fat tissue (e.g. in cell wall and cytoplasm), the fast lean tissue (e.g. visceral organs) and slow lean tissue (e.g. skeletal muscle).

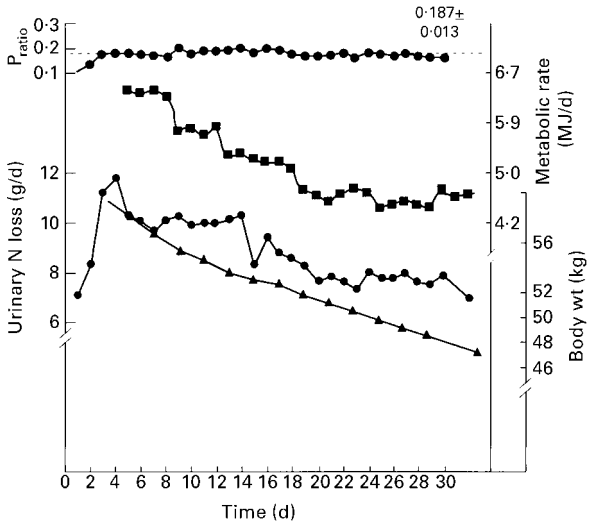

Fig. 2. Diagram showing the relative constancy of the fraction of energy mobilized from protein (P_{ratio}) during the course of fasting in the subject (Mr Levanzin) studied by Benedict (1915). Between day 4 and day 31 of the fast the mean and standard deviation of the P_{ratio} in this subject was 0·187 and 0·013. Data are reproduced from Henry *et al.* (1988); see p. 340.

and hence support the hypothesis of Payne & Dugdale (1977) that the control of partitioning is an individual characteristic.

To gain further insights into the biological significance of these concepts in body composition regulation during food deprivation and refeeding, we (Dulloo *et al*. 1996) recently tested the concepts of inter-individual variability and intra-individual constancy of energy-partitioning by re-visiting the classic Minnesota experiment (Keys *et al*. 1950), a unique longitudinal study of semi-starvation and refeeding which has a reputation for being meticulous in detail, and conducted under tightly controlled conditions of diet and lifestyle. From the individual data on changes in body fat and fat-free mass (FFM) in the thirty-two men in the Minnesota study, we calculated the fraction of body energy either lost as protein during semi-starvation or deposited as protein during refeeding, i.e. an integrated P_{ratio}, using the equation:

$$P_{ratio} = \frac{1}{1 + \alpha \times \frac{\Delta FAT}{\Delta FFM}}, \qquad (1)$$

where $\Delta FAT : \Delta FFM$ is the ratio of changes in fat mass and FFM, and where $\alpha = 9{\cdot}05$, the ratio of energy equivalents for fat mass (38·9 MJ/kg) and FFM (4·3 MJ/kg); the proportion of protein in FFM is taken as 23 % (Forbes *et al*. 1953, 1956) and the energy density for endogenous protein is taken as 18·6 MJ/kg (Livesey & Elia, 1988).

Within this population of thirty-two young Caucasian men, the P_{ratio} values were found to be highly variable during both semi-starvation and refeeding. Although the P_{ratio} during refeeding was found to be lower than the P_{ratio} during semi-starvation, they were nonetheless strongly correlated. By applying both statistical and numerical approaches to these data on P_{ratio} assessed during two phases of weight change (loss and recovery), we were able to show that the lower P_{ratio} during refeeding is not due to a shift in the individual's Pc, but can be attributed to other mechanisms operating via the suppression of thermogenesis in response to severe fat depletion, with the energy thus conserved being directed specifically towards accelerating fat (and not protein) recovery (Dulloo *et al*. 1996). While this finding that the Pc of the Minnesota men during semi-starvation was conserved during refeeding also supports the Payne & Dugdale (1977) hypothesis that the partitioning between protein and fat is a characteristic of the individual, it also argues against their stipulation that the P_{ratio} during refeeding must be equal to the P_{ratio} during starvation (Dulloo, 1998). Based on our reanalysis of the Minnesota experiment, it is clear that the notion of intra-individual constancy in fuel partitioning is valid only if, as shown schematically in Fig. 1, a distinction is made between: (a) the control of partitioning between protein and fat, which refers to a control system that underlies the Pc of the individual, and (b) the P_{ratio}, which, defined as the fraction of energy mobilized or deposited as protein, refers to the integrated outcome of several control systems (the control of partitioning being only one) that operate to regulate body composition. Taken together, these findings from these classic human studies of prolonged fasting and semi-starvation–refeeding, therefore lend support to the notions of inter-individual variability and intra-individual constancy within the context of an autoregulatory control of energy-partitioning between protein and fat.

Initial adiposity as a determinant of inter-individual variability in P_{ratio}

From a historical perspective, it has long been known that the P_{ratio} during starvation is influenced by the pre-starvation level of adiposity. As Elia (1991) reminded us in his elaborate review on the effect of starvation on protein–energy interrelationships, an inverse association between the contribution of protein as fuel during starvation and the initial degree of fatness was observed from inter-species studies nearly a century ago, notably by Voit (1901). However, interest in the nature and functional importance of this relationship in human subjects is relatively recent. First, on the basis of a meta-analysis of data on the composition of weight loss in lean and obese patients on low-energy diets, Forbes (1987) reported an inverse curvilinear relationship between the proportion of weight loss as FFM and the initial fat content. A number of other workers have added more data points to the original set analysed by Forbes, and they have confirmed the curvilinear form of this relationship, whether the proportion of weight loss as FFM is plotted against total body fat (Prentice *et al*. 1991) or against the BMI (Ferro-Luzzi *et al*. 1994). Second, in their re-analysis of human studies of prolonged fasting, Henry *et al*. (1988) reported that the fasting P_{ratio} declines in an approximately linear manner with increasing adiposity. However, in his critical review on this topic, Elia (1991) pointed out that the greatest changes in fasting P_{ratio} occur when the variations in BMI (or in estimated initial percentage fat) are at the lower end of the scale, and argued that the relationship is indeed curvilinear on the basis of these experimental data, as well as from

theoretical considerations. Third, in an attempt to identify statistically the predictors of inter-individual variability in energy-partitioning among the men in the Minnesota experiment, we correlated the P_{ratio} of the Minnesota men during semi-starvation against all available data on their physical characteristics during the baseline (pre-starvation) period, including height, sitting height, cormic index (sitting height/height), body weight, BMI, fat, FFM, percentage fat, abdominal circumference, arm circumference and age. Several of these variables were correlated with the P_{ratio}, but in a stepwise regression analysis the initial percentage fat was the only predictor of the P_{ratio} that remained, while other predictors (including the next best correlate, total fat) dropped out (Dulloo *et al*. 1996). As shown in Fig. 3, the relationship between the initial percentage fat (FAT_0) and the P_{ratio} during semi-starvation is an inverse exponential, with an r^2 value of 0·71, suggesting that about 70 % of the variation of P_{ratio} could be explained by the variation in percentage fat before food deprivation in this population sample.

Limitations of meta-analyses for studying 'biological' variability in P_{ratio}

In a previous attempt to find out whether the strength of the relationship between initial adiposity and the P_{ratio} would improve further if we included data from individuals with higher percentage fat in the regression analysis, we pooled the Minnesota data based on normal-weight Caucasian subjects with those derived from obese Caucasian subjects who also underwent prolonged semi-starvation (Passmore *et al*. 1958; Strong *et al*. 1958) or total starvation (Henry *et al*. 1988), and for whom we estimated pre-starvation percentage fat values on the basis of weight, height and age using the algorithm of Heitmann (1990). The correlation coefficient for such a meta-analysis was reported to be an 'improvement' over that based entirely on the Minnesota data (r^2 0·84 *v*. r^2 0·7) (Dulloo *et al*. 1996; Dulloo, 1997). However, we were made to realize, retrospectively, by statistician colleagues, that since the distribution of data constituted two distinct clusters, with the Minnesota data in the range 6–25 % fat and the obese data in the range 35–55 % fat, the gap in data distribution (between 25 and 35 % fat) introduces a statistical error which will tend to increase the correlation coefficient (Armitage & Berry, 1987). In other words, the 'improvement' in the regression coefficient in our meta-analysis may be interpreted as a statistical artifact. Furthermore, in meta-analyses of data from studies of partial and/or total starvation reported so far (Forbes, 1987; Henry *et al*. 1988; Elia, 1991; Prentice *et al*. 1991; Ferro-Luzzi *et al*. 1994; Dulloo *et al*. 1996) the importance of initial adiposity *per se* as a determinant of biological variability in P_{ratio} is confounded by several variables inherent in such meta-analyses, such as sex, age, ethnicity, diet composition, degree and duration of starvation, exercise levels, types of obesity (variable abnormalities in metabolic and endocrine profiles), and by large errors in the estimation of percentage body fat from equations based on weight and height, or in the use of different methodologies in the measurement of body fat.

Validity of the Minnesota data for studying 'biological' variability in P_{ratio}

By contrast, the analysis of the Minnesota experiment in its own right, by virtue of its design *vis-à-vis* selection criteria of subjects (on the basis of physical, clinical, biochemical and psychological tests), more controlled conditions of diet and lifestyle before and during the experiment, and with body fat being measured (by hydrodensitometry) rather than estimated, bypasses many of these previously-mentioned limitations. For example, the thirty-two individuals (all Caucasians, men, and of similar age) were studied simultaneously under the same environmental (laboratory) conditions, and measurements of body composition were made using the same methodology. They were subjected to the same semi-starvation diet, and lost approximately 25 % of initial body weight over same time period (i.e. same starvation stress). Their day-to-day pattern of physical activity was controlled for the most part of the study, and no one among the Minnesota men was subjected to the type of intense physical activity or resistance training that would provide any major anabolic drive on muscle mass. Furthermore, the subjects were semi-starved (and not fasted) and consumed a balanced restricted diet with the carbohydrate level either adequate or nearly adequate *vis-à-vis* the obligatory glucose requirements of the brain and other vital tissues, such that the protein-sparing associated with the brain's shift in fuel utilization partially towards ketone bodies was likely to be minimal and of short duration. It may still be argued that this analysis of the Minnesota data is limited by lack of individuals with higher percentage fat, and hence by a wider range of adiposity for the purpose of regression analysis. However, this 'limitation' needs to be weighed against the fact that the inclusion of overtly obese individuals in such an analysis would also have increased the risk for blurring our understanding of normal physiology of body composition regulation with pathophysiological conditions associated with more marked obesity, such as insulin resistance, and other disturbances in metabolic–hormonal profiles. Since in the present paper, the autoregulatory control of energy partitioning is examined first and foremost from a standpoint of normal physiology, we take the view that the population sample of healthy men in the Minnesota experiment, with a normal distribution of percentage body fat ranging from the very lean (6 % fat) to the mildly obese (25 % fat) provides a sufficiently large (4-fold) variability in initial adiposity for this purpose, particularly when it is within this range of 6–25 % body fat that much of the steep part of the exponential curve between initial adiposity and the P_{ratio} lies (Fig. 3). Bearing in mind the well-controlled and similar experimental conditions of diet and lifestyle to which the thirty-two men in the Minnesota study were exposed, it is likely that the large inter-individual variability in P_{ratio} observed during the semi-starvation phase of the Minnesota experiment reflects essentially 'biological' variability in P_{ratio}. In this context, the reanalysis of its data underlines the critical importance of initial percentage fat as an internal determinant of human variability in fuel partitioning during starvation.

Fundamental questions

There are, however, two fundamental questions that arise from the relationship between initial percentage fat and P_{ratio} shown in Fig. 3.

(i) In the context of the present paper whereby the partitioning between protein and fat is viewed to be under an autoregulatory control system of central importance in regulating body composition, and the well-known association between the initial percentage fat and the duration of starvation (hence the length of survival) (Elia, 1991), the challenge resides in providing a mechanistic explanation for the way the initial percentage fat, via the control of energy partitioning between protein and fat, confers maximal survival autonomy during food scarcity. In other words, what could be the biological significance (in terms of survival value) of this strong relationship between initial percentage fat and the P_{ratio} shown in Fig. 3?

(ii) It is also clear from Fig. 3 that the initial percentage fat does not explain all the variance in P_{ratio}. For a given initial percentage fat value, there is still a 2-fold variability in the P_{ratio}, and the residual variance after adjustment for variability in the initial percentage fat (SD 0·044) is still about half of the total variance in P_{ratio} (SD 0·082). In other words, the initial percentage fat explains only about half of the total variance in P_{ratio} among the Minnesota men. It is possible that this residual variance reflects, at least in part, methodological errors, and/or some undefined externally-induced source of variability among the Minnesota men. However, on the basis of our previously-reported analysis of P_{ratio} during semi-starvation and refeeding (Dulloo *et al*. 1996) showing that the Pc of the individual was conserved during these two opposing phases of weight changes, such that the residual variance during weight loss was also conserved during weight recovery, the hypothesis is put forward here that much of the residual variance in this relationship between P_{ratio} and initial percentage fat also represents biological variability in fuel partitioning. The question then arises as to what could be the determinant(s) and biological significance of this variability in P_{ratio} that is independent of the initial percentage fat?

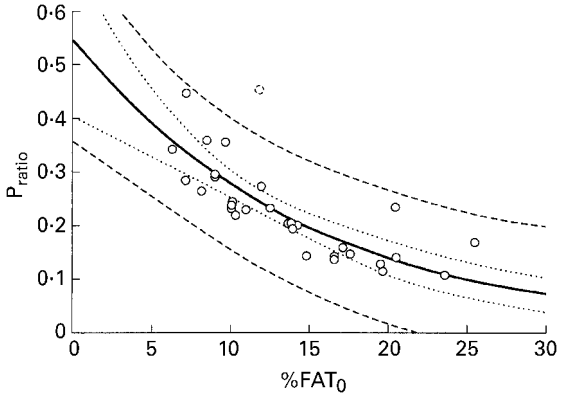

Fig. 3. Exponential relationship between the fraction of energy lost as protein during semi-starvation (P_{ratio}) and the initial percentage body fat (FAT_0) in the Minnesota experiment (*n* 32) (Keys *et al.* 1950). The data for initial body fat (range 6–25 %) follow a normal distribution, with an almost 3-fold variability between the 10th percentile value (7·4 %) and the 90th percentile value (20·5 %), and with the 50th percentile (median) value being 13·7 %. For the regression (*n* 32, r^2 0·6, $P < 0·001$), the 95 % CI are within the inner dotted lines, whereas the predictive intervals are within the outer broken lines. Only one data point (○) lies well outside the prediction lines, and it has been omitted in subsequent analyses; its omission in the regression analysis improves the r^2 from 0·6 to 0·7 ($P < 0·001$). The data for P_{ratio} have been calculated using equation 1 (see p. 341) from the Minnesota experiment data on body fat and fat-free mass, corrected for excess hydration and for the relative bone mass at the end of semi-starvation on the basis of formula derived on a sub-group of the Minnesota men (*n* 17) in whom extracellular fluid space was determined by the thiocyanate dilution technique (Keys *et al.* 1950). The exponential fit was performed using the computer software Peakfit Version 2.0 (Jandel Scientific Software, San Rafael, CA, USA).

Plan of remaining part of this paper

To address these questions, we first conduct a quantitative analysis of the relationship between initial body composition ($FAT_0 : FFM_0$) and the composition of weight loss ($\Delta FAT : \Delta FFM$). On the basis of the results of this analysis, a number of concepts are brought together, further developed, and integrated to provide possible answers to the questions posed earlier, and hence to explain the biological significance of the relationship between initial percentage fat and P_{ratio}, as well as that of the residual variability in this relationship. These concepts are then used in the construction of a mathematical model for predicting the Pc of the individual. The various applications of the model in explaining inter-individual variability in fuel partitioning during early and prolonged starvation, as well as in the maximum percentage weight loss from starvation are then discussed.

Quantitative analysis of the relationship between initial percentage fat and P_{ratio}

The basic concepts and assumptions underlying this analysis (as discussed in detail earlier) are that (i) the Pc of the individual is a constant, independent of time during starvation, and that (ii) in the Minnesota experiment, the integrated P_{ratio} during semi-starvation is a proxy of the Pc of the individual, given that the design of this unique study was such that internal or external perturbations on the fat and/or protein compartments were likely to be absent or of negligible quantitative importance when integrated over the 24-week study period.

Relationship between initial body composition and the composition of weight lost

Specifically, we have explored how the P_{ratio} and the initial percentage fat of the Minnesota men (in Fig. 3) are related quantitatively by expressing both the independent and the dependent variables in the same form, i.e. in terms of fat and FFM, corrected for excess hydration and relative bone mass (Keys *et al*. 1950). Using equations 2 and 3, the P_{ratio} is converted into a ratio of the loss in body fat to that in FFM, i.e.

as $\Delta FAT : \Delta FFM$, while the initial percentage fat is converted into a ratio of initial fat to that of FFM, i.e. as $FAT_0 : FFM_0$.

$$\frac{\Delta FAT}{\Delta FFM} = \frac{1}{\alpha} \times \left(\frac{1}{P_{ratio}} - 1 \right), \quad (2)$$

$$\frac{FAT_0}{FFM_0} = \frac{(\%FAT_0)}{(100 - \%FAT_0)}, \quad (3)$$

where $FAT_0 = \%FAT_0 \times W_0$, (3a)

$$FFM_0 = (100 - \%FAT_0) \times W_0, \quad (3b)$$

and where $\%FAT_0$ is the initial percentage body fat, and W_0 is the initial body weight.

A plot of the composition of weight loss ($\Delta FAT : \Delta FFM$) against the initial body composition ($FAT_0 : FFM_0$) is presented in Fig. 4, and shows that (i) all the data points lie above the line of identity and (ii) the relationship between $\Delta FAT : \Delta FFM$ and $FAT_0 : FFM_0$ is linear. The 95 % confidence and predictive intervals of this relationship are shown in Fig. 4, and the statistics for the regression model are provided in the legend to this figure.

Interpreting the relationship in Fig. 4

If it is accepted that the energy density of the FFM lost (after correction for excess hydration and for the relative bone mass) is close to the energy density of the initial FFM, then the following statements can be made:

(i) the fact that the data points do not lie on the line of

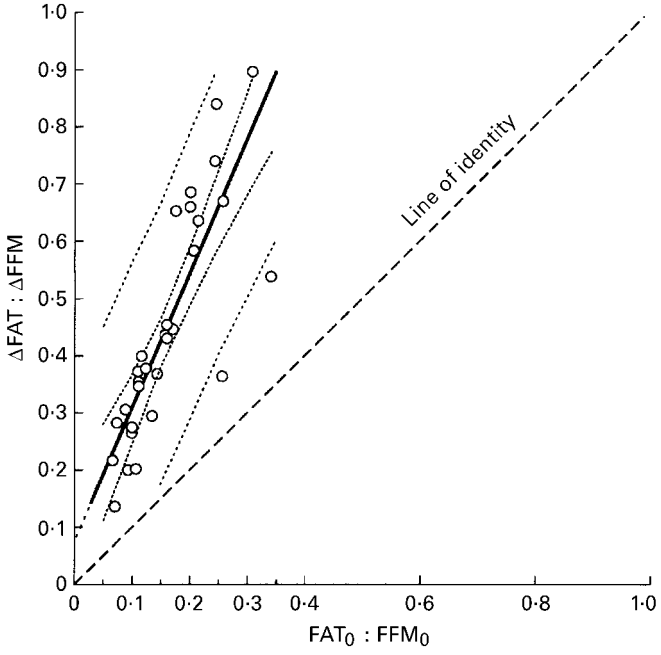

Fig. 4. Linear relationship between the initial body composition (FAT_0 : fat-free mass (FFM_0)) and the composition of weight loss ($\Delta FAT : \Delta FFM$) among the men subjected to 24 weeks of semi-starvation in the Minnesota experiment (*n* 31; r^2 0·7, $P < 0·001$). The data for $\Delta FAT : \Delta FFM$ are the values for change in body fat and FFM over the entire 24 weeks of semi-starvation. The data for $FAT_0 : FFM_0$ (range 0·06–0·34) follow a normal distribution, with a 3-fold variability between the 10th percentile value (0·08) and the 90th percentile value (0·26), and with the 50th percentile (median) value being 0·16. For the regression, the 95 % CI are within the inner dotted lines, whereas the predictive intervals are within the outer dotted lines. The significance of the overall linear model is high (*F* value 55·5, $P < 0·0001$), with a significant slope (*b*) of 2·29 (SD 0·31) ($P < 0·0001$), and a non-significant constant intercept (*a*) of 0·078 (SD 0·055) ($P = 0·16$). It is to be noted that the statistical treatment of data that are found within the predictive intervals (i.e. if the two possible outliers are omitted) reveals that the significance of the overall linear model is even higher (*F* value 240, $P < 0·0001$), with a significant slope (*b*) of 3·15 (SD 0·20) ($P < 0·0001$), and further confirms that the constant intercept (*a*) of –0·033 (SD 0·034) ($P = 0·33$) is not significantly different from zero. The broken diagonal line represents the line of identity between the two ratios. The simple linear-regression model was applied using the computer software programme STATISTIK (version 4.0; Analytical Software, St Paul, MN, USA).

identity suggests that the composition of the weight lost ($\Delta FAT : \Delta FFM_0$) is not equal to the initial body composition ($FAT_0 : FFM_0$);

(ii) the position of the data points well above the line of identity illustrates the fact that the proportion of fat to protein mobilized ($\Delta FAT : \Delta FFM$) during food deprivation is greater than the proportion of fat to protein present in the body before food deprivation ($FAT_0 : FFM_0$);

(iii) the linearity of this relationship underlies the fact that the ratio of these two proportions (the slope), being a constant, is independent of the initial degree of adiposity of the individuals in this population sample.

Based on the assumptions (discussed earlier) that for each of the Minnesota men, the ratio $\Delta FAT : \Delta FFM$ calculated over the 24 weeks of semi-starvation represents the outcome of a control system underlying his Pc, and that the latter remains a constant over time during food deprivation, then by extrapolation, these derivations from Fig. 4 would imply that:

(i) since as indicated in the first two derivations above, i.e. $\Delta FAT : \Delta FFM > FAT_0 : FFM_0$, the body composition of a given individual changes continuously towards a leaner composition during the course of starvation. In other words, if the body composition at a given time (t) during starvation is $FAT_t : FFM_t$, then as weight is being lost with time, the ratio $FAT_t : FFM_t$ decreases relative to the initial ratio $FAT_0 : FFM_0$, and consequently, the fat compartment is virtually depleted well before that of the protein (FFM) compartment. Such a contention is supported by autopsy findings indicating that in human subjects who died of 'uncomplicated' starvation, the fat depots are virtually absent whereas the loss in fresh organs (i.e. lean tissue mass) is less than 50 % (Meyers, 1917; Keys *et al*. 1950; Elia, 1991), and

(ii) the fraction of protein compartment lost when the fat compartment is virtually depleted may be independent of the initial body composition ($FAT_0 : FFM_0$) or initial percentage body fat, a contention that can be shown mathematically in two steps as outlined here.

Step I. Since the value of the intercept in the relationship between $\Delta FAT : \Delta FFM$ against $FAT_0 : FFM_0$ in Fig. 4 is not significantly different from 0 (see legend for statistics), the regression line is assumed to pass through the origin, and it can be described as:

$$\frac{\Delta FAT}{\Delta FFM} = m \times \frac{FAT_0}{FFM_0}, \tag{4}$$

where m is the slope.

Step II. Since when the fat compartment is virtually depleted, $\Delta FAT = FAT_0$, the fraction of the protein compartment lost at this 'theoretical' time-point of total depletion of the fat compartment ($\Delta FFM_{zeroFAT} : FFM_0$) can be shown to be independent of FAT_0 or FFM_0 by replacing ΔFAT by FAT_0, and ΔFFM by $\Delta FFM_{zeroFAT}$ in equation 4 as follows:

$$\frac{FAT_0}{\Delta FFM_{zeroFAT}} = m \times \frac{FAT_0}{FFM_0},$$

i.e.

$$\frac{\Delta FFM_{zeroFAT}}{FFM_0} = \frac{1}{m} \tag{5}$$

Taken together, this 'quantification' of the exponential relationship between P_{ratio} and initial percentage fat of the Minnesota data, presented in Fig. 4 and equation 5, therefore reveal that when body fat is virtually depleted, the fraction of FFM lost is, in our model, independent of the initial body composition ($FAT_0 : FFM_0$ or $\%FAT_0$).

The validity of the demonstration rests on the assumption that the straight line in Fig. 4 passes through zero. Although the statistical analysis supports a null hypothesis about the intercept, i.e. the intercept is not significantly different from zero, this does not, however, demonstrate that the intercept is zero. Consequently, one could entertain the possibility for this set of data (and other data sets) that the intercept may not be zero. If the alternative assumption (that the intercept is not zero) is put forward, then equation 5 will have to be rewritten as follows:

$$\frac{\Delta FFM_{zeroFAT}}{FFM_0} = \frac{1}{\left(a \times \frac{FFM_0}{FAT_0} + m\right)},$$

where a is the value for the intercept.

If for the present set of data, the value of a is taken as 0·078 (SD 0·055) ($P = 0{\cdot}16$, NS) and the slope m as 2·29 (as indicated in the legend to Fig. 4), and if the $FFM_0 : FAT_0$ value for human adults is taken as ranging from 3 (30 % body fat) to 12 (5 % body fat), then this version of equation 5 indicates that values for the ratio $\Delta FFM_{zeroFAT} : FFM_0$ would correspondingly range over about 30 %. If the value of the intercept is taken to be that for the upper confidence limit of the estimation (i.e. 0·18), then the corresponding range of $\Delta FFM_{zeroFAT} : FFM_0$ would be 40 %. On the other hand, if (as also indicated in the legend to Fig. 4) the two possible outliers are omitted in the analysis, in which case the value of a is –0·033 (SD 0·034) ($P = 0{\cdot}33$, NS), with an upper confidence limit of 0·035 and the slope m is 3·15, then for the same range of $FFM_0 : FAT_0$ values for human adults (i.e. 3–12), the corresponding range for $\Delta FFM_{zeroFAT} : FFM_0$ values would vary between 8 and 10 %. Thus, on the basis of the values of the intercept of this data set, when the body fat is virtually depleted, the fraction of FFM lost could vary by as much as 30–40 % depending on the initial body composition, or by as little as 8–10 %. In the present state of knowledge, one cannot safely accept nor reject any one of the possibilities that the actual value for the intercept is zero, 0·078 or –0·033 in this set of data, let alone to make the much wider assumption for all other data sets (i.e. in a general model). However, the principle of parsimony in modelling, coupled with statistical analysis indicating that the intercept is not significantly different from zero, guides us to choose the assumption (or to hypothesize) that the intercept is zero in the construction of our model. If future evidence suggests otherwise, then the model will have to be adapted to take into account the new parameters, whose biological significance would then need to be studied.

Concepts underlying optimal utilization of two energy reserves

On the basis of these new findings from the Minnesota data, coupled with autopsy findings that the fat depots are virtually absent in human subjects who have died of uncomplicated starvation (Meyers, 1917; Keys *et al*. 1950; Elia, 1991), we describe here the process of 'concept building' that provides possible explanations concerning the biological significance of (a) the relationship between initial percentage fat and the P_{ratio} in Fig. 3, and (b) the residual variability in this relationship.

Concept I

By virtue of its independence from the initial body composition, this value $1/m$, the fraction of FFM lost when fat mass is virtually depleted (in equation 5), could thus represent a critical threshold in the amount of protein that can be mobilized without irreversible or lethal consequences, and could hence be considered to represent the fraction of the total protein compartment that is 'dispensible'. In this context, it represents the fraction of the total protein compartment that functions as an energy reserve. This 'protein-reserve' fraction (r_p) can be described as:

$$r_p = \frac{\Delta FFM_R}{FFM_0}, \quad (6)$$

where ΔFFM_R is the protein-reserve component of the total protein compartment FFM_0.

By analogy, there is a component in the fat compartment that is indispensible for life (e.g. membrane phospholipids, brain and nerve lipids). The fraction of the fat compartment that is 'dispensible' (essentially stored triacylglycerols) is hence considered as the fraction of the total fat compartment that functions as an energy reserve. This 'fat-reserve' (r_f) fraction can be described as follows:

$$r_f = \frac{\Delta FAT_R}{FAT_0}, \quad (7)$$

where ΔFAT_R is the fat-reserve component of the total fat compartment FAT_0.

From a historical perspective, the concept of 'dispensible' and 'indispensible' components of FFM and/or fat compartments is not new, and their origins can be traced to the era soon after Voit's experiments on starving animals (Keys *et al*. 1950). In more recent decades, they have frequently been used in one form or another in the construction of models of starvation, notably by Payne & Dugdale (1977), Le Maho *et al*. (1988) and Elia (1991). However, the mathematical articulation of these concepts, as described earlier, is new. Although the body's fat and protein-energy compartments in these equations are denoted by fat mass and FFM by necessity of the data available, it needs to be emphasized that conceptually, the protein-energy compartment comprises protein in all tissues (including adipose tissues), and the fat-energy compartment comprises lipids in all tissues (including inter-muscular triacylglycerol stores, and membrane lipids).

Concept II

If the indispensible (non-reserve) fat component remains intact during starvation, death will occur if the fat-reserve is depleted even if the protein-reserve is far from depleted. This contention is based upon the notion put forward by Leiter & Marliss (1982) that fat is required to fuel the energy cost of hepatic gluconeogenesis, and that inadequate fatty acid supply will lead to a limited glucose supply to obligate glucose-consuming organs, despite adequate supplies of gluconeogenic substrates. Consequently, in the face of chronic food deprivation, the best survival strategy is for the individual to meet his fuel needs by a co-ordinated withdrawal of energy from these two energy reserves, such that both the 'fat-reserve' component and the 'protein-reserve' component reach complete depletion simultaneously.

This concept II contrasts with the Payne & Dugdale (1977) concept about the operation of partitioning of tissues during starvation which suggests that when the storage fat is completely depleted, the P_{ratio} values 'switch' from being a characteristic of the individual and assume the proportion of total energy derived from the combustion of lean tissue. This Payne–Dugdale concept has origins with the idea of a 'premortal rise in N excretion' (reflecting a degenerating and irreversible protein utilization) following Voit's experiments on starving animals (Keys *et al*. 1950). However, as Le Maho *et al*. (1988) have pointed out, the data in the literature on several animal species (with the exception of the emperor penguin (*Aptenodytes forsteri*)) are contradictory, with some data showing no increase in protein utilization, and others indicating that animals could harmlessly be refed. There are also no data in the literature that support the existence of a rise in protein utilization in lean or obese human subjects (Le Maho *et al*. 1988), and in a review on prolonged human starvation, Grande (1964) came to the conclusion that there is no serious argument for the existence of a premortal increase in N excretion. On the other hand, concept II that both the 'fat-reserve' component and the 'protein-reserve' component reach complete depletion simultaneously is consistent with the conclusion about the optimal strategy for prolonged survival derived by Elia (1991) from the hypothetical scheme of fuel availability, composition of weight loss, and survival time in a typical lean man and an obese man twice ideal body weight.

Concept III

Inter-individual variability in r_p, in r_f or in both these two reserve fractions could explain the residual variance in the relationship between P_{ratio} and initial percentage fat. Similar concepts can also be traced from the work of Le Maho *et al*. (1988) and Elia (1991).

A mathematical model of energy-partitioning during starvation

By integrating these concepts of dual energy reserves emptying simultaneously during starvation (i.e. the protein-reserve compartment, ΔFFM_R, reaching total depletion at the same time as the fat-reserve compartment, ΔFAT_R) into the

calculations of the P_{ratio}, i.e. by replacing ΔFFM and ΔFAT in equation 1 by ΔFFM_R ($=r_p \times FFM_0$) from equation 6 and by ΔFAT_R ($=r_f \times FAT_0$) from equation 7 respectively, we obtain a general equation that defines the theoretical Pc of an individual as follows:

$$Pc = \frac{1}{1 + \alpha \frac{r_f}{r_p} \times \frac{FAT_0}{FFM_0}}. \quad (8)$$

Using equation 3, equation 8 becomes:

$$Pc = \frac{1}{1 + \alpha \frac{r_f}{r_p} \times \frac{\%FAT_0}{(100 - \%FAT_0)}}, \quad (9)$$

or

$$Pc = \frac{100 - \%FAT_0}{100 - \left(1 - \alpha \times \frac{r_f}{r_p}\right) \times \%FAT_0}, \quad (10)$$

where $r_f : r_p$ is the ratio of the fat and FFM energy-reserve fractions.

This model can be used to study inter-individual variability in Pc, by varying the initial percentage body fat and the ratio $r_f : r_p$, i.e. the three factors which together confer the Pc of the individual. This is illustrated in Fig. 5, where the value of $\alpha \times r_f : r_p$ has been varied between 15 and 40 (corresponding to $r_f : r_p$ values between 1·66 and 4·42). It is also shown in Fig. 6 that the model, with an $\alpha \times r_f : r_p$ value of 25·4 (corresponding to an $r_f : r_p$ value of 2·8), produces a best fit which is superimposable to the exponential curve previously obtained for the relationship between P_{ratio} and initial percentage fat among the Minnesota men in Fig. 3. As a whole, this model provides a tool which can be used to study inter-individual variability in fuel partitioning in response to starvation. It can in fact be used to examine the relative importance of each of these variables in determining the Pc of the individual in the Minnesota experiment. This first application of the model, together with several others, is described now.

Applications of the model

I. Relative importance of r_p and r_f as determinants of variability in partitioning characteristic

This mathematical model of partitioning thus embodies not only the concept that the Pc of the individual during starvation is determined by the pre-starvation ratio fat : protein compartments ($\%FAT_0$), but also the new concepts of a dual energy-reserve ($r_f : r_p$) emptying simultaneously. However, it can be shown in steps 1–4 here that variability in the fat reserve fraction (r_f) is essentially also a function of $\%FAT_0$, such that inter-individual variability in Pc is determined only by variability in $\%FAT_0$ and in r_p, i.e. in the initial percentage fat and in the protein-reserve fraction.

Step 1. It can be assumed that the 'indispensible' fat component is practically independent of body size and body composition in the adult human being, since it is constituted (a) from the brain whose size (and hence lipid content) is independent of body size in the adult man, and (b) from structural lipids in nerves and cell membranes whose

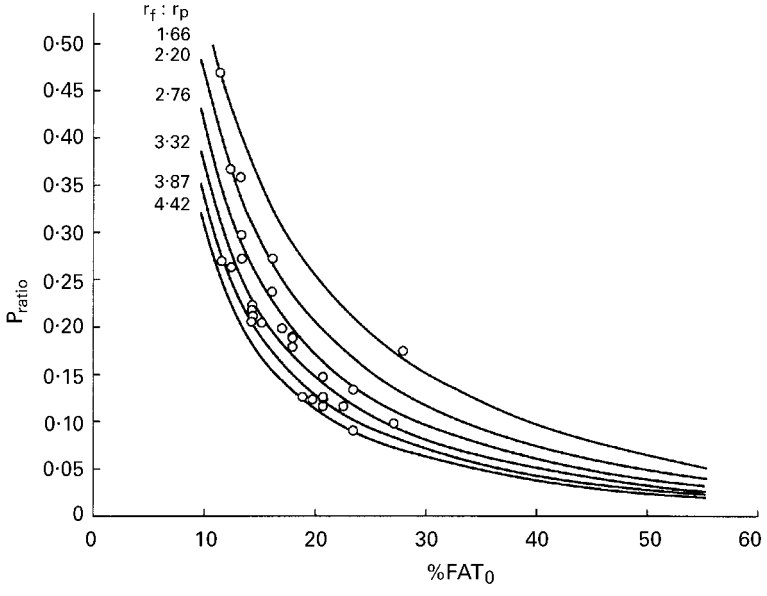

Fig. 5. Model prediction for the partitioning characteristic (Pc) as a function of initial percentage body fat ($\%FAT_0$), with variable values for the ratio fat-reserve fraction : protein reserve fraction ($r_f : r_p$) using equation 10 (see above). The theoretical Pc (solid curves) are superimposed over the data for fraction of energy mobilized as protein (P_{ratio}) of the Minnesota men (Keys *et al.* 1950), and share the same Y-axis. Note that the P_{ratio} data of the Minnesota men are found within theoretical Pc curves generated from $\alpha \times r_f : r_p$ values between 15 and 40, with $\alpha = 9{\cdot}05$ (this corresponds to $r_f : r_p$ values in the range of 1·66–4·42).

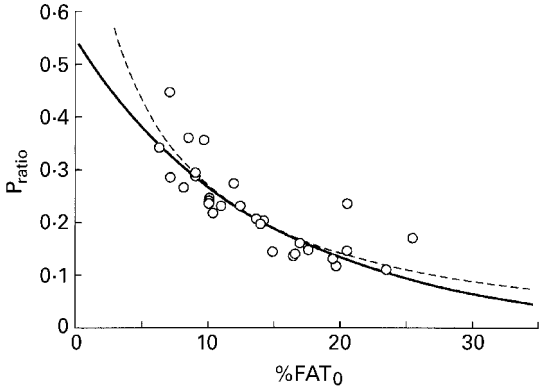

Fig. 6. Comparison of the best fit of the model prediction of the partitioning characteristic (– – –, r^2 0·73; $r_f : r_p$ 2·8, α 9·05) as a function of initial percentage body fat (%FAT_0) with the actual exponential relationship (——, r^2 0·71) observed between data for the fraction of energy mobilized as protein (P_{ratio}) of the Minnesota men and %FAT_0. Note that both curves are almost superimposable when %FAT_0 is greater than 5% (the lower limit of percentage body fat in a healthy population), and that both the predicted and actual relationships yield similar values for r^2, i.e. 0·7.

variation with body size is likely to be quantitatively small. Consequently, the absolute amount of fat that is indispensible (non-reserve fat) can be considered to be a constant in such a population sample.

Step 2. Based on autopsy studies reporting that in men who died of starvation, the fat depots are virtually absent (Meyers, 1917; Keys *et al*. 1950), and that the remaining non-reserve fat is approximately 1 kg (Elia, 1991), the fat-reserve (ΔFAT_R) and fat reserve fraction (r_f) in the Minnesota men can therefore be described as:

$$\Delta FAT_R = FAT_0 - 1,$$

$$r_f = \frac{FAT_0 - 1}{FAT_0}. \qquad (11)$$

Step 3. Equation 11 shows that r_f is a function of FAT_0, but given the very strong correlation between %FAT_0 and FAT_0 (in the Minnesota men, r 0·98), r_f is thus a function of %FAT_0.

Step 4. Since the variability in r_f is essentially reflected in the variability in %FAT_0, it follows that the Pc has only two determinants: %FAT_0 and r_p, namely:

$$Pc = f\left(\frac{1}{\%FAT_0}, r_p\right). \qquad (12)$$

Thus the model, via equations 10 and 12, helps to explain the plausibility of the importance for r_p in the residual variance in the relationship between P_{ratio} and initial percentage fat (Fig. 3). It predicts that individuals with the highest P_{ratio} values are those with low initial percentage fat and high r_p (i.e. lean individuals with a large protein-reserve fraction), whereas individuals with the lowest P_{ratio} values are those with high initial percentage fat and low r_p (i.e. obese individuals with a small protein-reserve fraction). Furthermore, the model suggests that the contribution of r_p to the variability in Pc is equally as important as that of the initial percentage fat. For example, if either %FAT_0 or r_p is increased by 2-fold in equation 10, the same variation in Pc is obtained.

Since r_f values of the Minnesota men can now be estimated

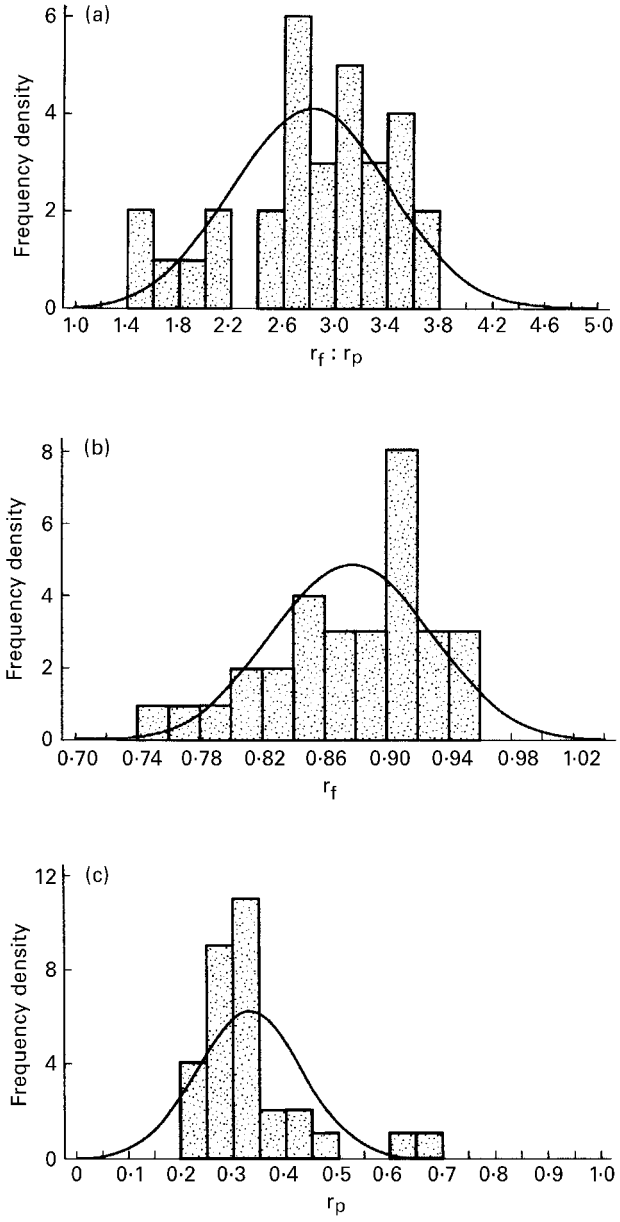

Fig. 7. Histograms showing frequency distributions of the Minnesota men (Keys *et al.* 1950) for (a) the ratio fat-reserve fraction : protein-reserve fraction ($r_f : r_p$; mean 2·81 (2SD 1·20)), (b) the fat-reserve fraction (r_f; mean 0·88 (2SD 0·10)) and (c) the protein-reserve fraction (r_p; mean 0·33 (2SD 0·20)). A normal curve is superimposed over each histogram for comparison. Although only the data for $r_f : r_p$ appear to be normally distributed, and those for r_p and r_f appear skewed, the application of the Wilk-Shapiro/Rankit normality statistical test shows that all the three variables ($r_f : r_p$, r_p and r_f) could conform to a normal distribution, with Wilk-Shapiro values of 0·94, 0·80, and 0·95 respectively; in the case of the data for r_p, if the two possible outliers are omitted, the Wilk-Shapiro value is improved from 0·80 to 0·95. For the assumptions and equations for calculating $r_f : r_p$, r_f and r_p, see p. 346.

using equation 11, their r_p values can be calculated from the ratio $r_f : r_p$, which in turn can be determined by applying the model (equation 10) to the data on P_{ratio} and initial percentage fat. This is shown here.

Step 1 : $$r_f = 1 - \frac{1}{FAT_0}.$$

$$\text{Step 2:} \qquad P_{ratio} = \frac{1}{1 + \alpha \times \dfrac{\Delta FAT}{\Delta FFM}}. \qquad (1)$$

$$\text{Step 3:} \quad r_p = \alpha\ r_f \times \frac{\%FAT_0}{(100 - \%FAT_0)} \Big/ \left(\frac{1}{P_{ratio}} - 1\right). \qquad (13)$$

The frequency distributions of values obtained for $r_f : r_p$ as well as for r_p and r_f are presented as histograms in Fig. 7, and each is compared with a normal distribution. Despite the apparent skewness of r_p and perhaps r_f also, application of statistical test reveals that all three parameters ($r_f : r_p$, r_p, r_f) could conform to a normal distribution (details of statistical test in the legend). The range of r_p for data points with 2SD of the mean is found to be between 0·13 and 0·53, and the r_p values corresponding to the 10th to 90th percentiles are 0·24 and 0·45 respectively. This large (at least 2-fold) variability in r_p contrasts with the much smaller variability (<25 %) in r_f, and even if the absolute amount of non-reserve fat was made to vary by 50 %, the variability in r_p would still be several-fold larger than in r_f.

II. Limits of the partitioning characteristic in a population of normal-weight individuals

The range of $r_f : r_p$ for data points with 2SD of the mean of the normal distribution curve is found to be between 1·6 and 4·0 (Fig. 7), suggesting that there is a 2·5-fold variability in $r_f : r_p$ for the range covering 95 % of this population sample based on individuals of normal body weight. The curves of Pc predicted by the model for this range of $r_f : r_p$ (variability essentially in r_p) are shown in Fig. 8, and could be considered to cover variability in P_{ratio} as a function of initial percentage fat for normal-weight men in general. At a given percentage fat in the zone of individuals with normal body weight and percentage fat, there is a 2-fold variability in Pc.

III. Extrapolation of the limits of the partitioning characteristic to a population of obese individuals

By extrapolation of these curves (which are based on the values for $r_f : r_p$ derived from a sample population of normal body weight) to greater values for percentage body fat (Fig. 8), it is found that the range of theoretical Pc predicted

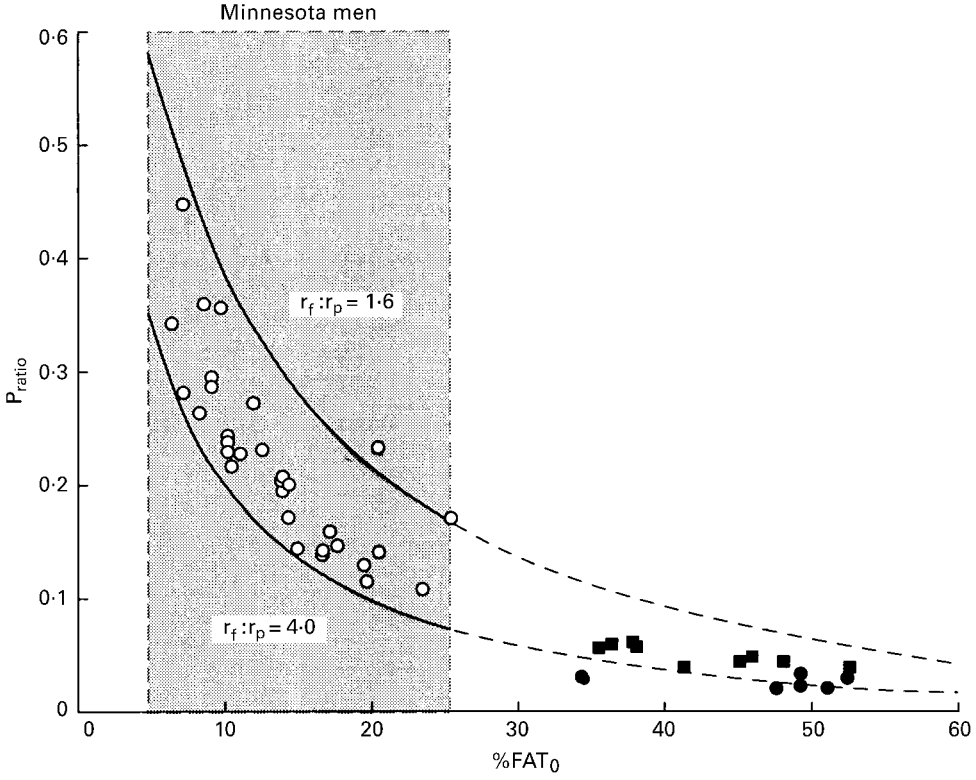

Fig. 8. Predictive limits (——) of the partitioning characteristic (Pc) within a Caucasian population with initial percentage body fat ($\%FAT_0$) in the normal range, i.e. the Minnesota men (Keys *et al.* 1950). These predictive limits are calculated from the mathematical model (equation 10) using the mean ± 2SD values for $r_f : r_p$ (i.e. 1·6 and 4·0) and hence cover 95 % of the $r_f : r_p$ values in this population sample (see Fig. 7, top histogram). These limits for Pc are shown to cover the measured values for fraction of energy mobilized as protein (P_{ratio}) (○) during semi-starvation in the Minnesota men (Keys *et al.* 1950). Extrapolation of these Pc limits to higher values of initial percentage body fat (----) also covers the data of P_{ratio} calculated for obese (Caucasian) individuals during prolonged semi-starvation (●) or prolonged fasting (■). The semi-starvation P_{ratio} values were calculated from energy and nitrogen balance during the last week of therapeutic low-energy-dieting lasting for 40–45 d in seven obese patients, and these data were derived from the work of Strong *et al.* (1958) and Passmore *et al.* (1958). The fasting P_{ratio} values were calculated from urinary nitrogen excretion and resting metabolic rate after at least 3 weeks of total starvation in nine obese patients, and these data were derived from the work of Ashley & Whyte (1961), Gilder *et al.* (1967*a*,*b*) and Owen *et al.* (1967); the assumptions inherent in the calculations of fasting P_{ratio} have previously been discussed by Henry *et al.* (1988) and by Elia (1991). For all these obese patients undergoing semi-starvation or fasting, we estimated their initial percentage body fat on the basis of their weight, height and age using the algorithm of Heitmann (1990).

by the model for the obese covers the actual P_{ratio} values determined in obese individuals in the zone of relative stability of their P_{ratio}, i.e. as discussed later, several weeks after the start of semi-starvation (Passmore *et al*. 1958; Strong *et al*. 1958) or total starvation (Ashley & Whyte, 1961; Gilder *et al*. 1967*a*,*b*; Owen *et al*. 1967). It is of interest to note from these predictive curves that individuals with initial percentage fat >35 fall on a relatively flat part of the exponential curve, such that in such obese individuals, variability in P_{ratio} is independent of initial percentage body fat, and hence simply due to variability in r_p.

IV. Partitioning characteristic and human variability in protein sparing

The concepts underlying the model of energy-partitioning have implications concerning human variability in the protein-sparing effect during the early phase of starvation, a phenomenon well described since the turn of the century by Benedict (1915), and whose biochemical and hormonal basis was laid down by Cahill and his collaborators in the late 1960's (Cahill, 1970). Since the fundamental assumption in this mathematical model is that the Pc of a given individual is stable over time during starvation, this would imply that in obese individuals, the partitioning mechanisms underlying their low Pc would be unable to provide sufficient protein as a substrate for gluconeogenesis and hence to meet their obligatory glucose requirements during the early phase of fasting as the glycogen stores become depleted. As the model predicts in Fig. 9, individuals with body fat >30 % would show a marked energy deficit between the brain's energy requirements (horizontal shaded zone) and the amount of energy derived from protein, entirely on the basis of the Pc (referred to as Δprotein-Pc), with the deficit being greater with lower values for r_p. Consequently, other mechanisms that

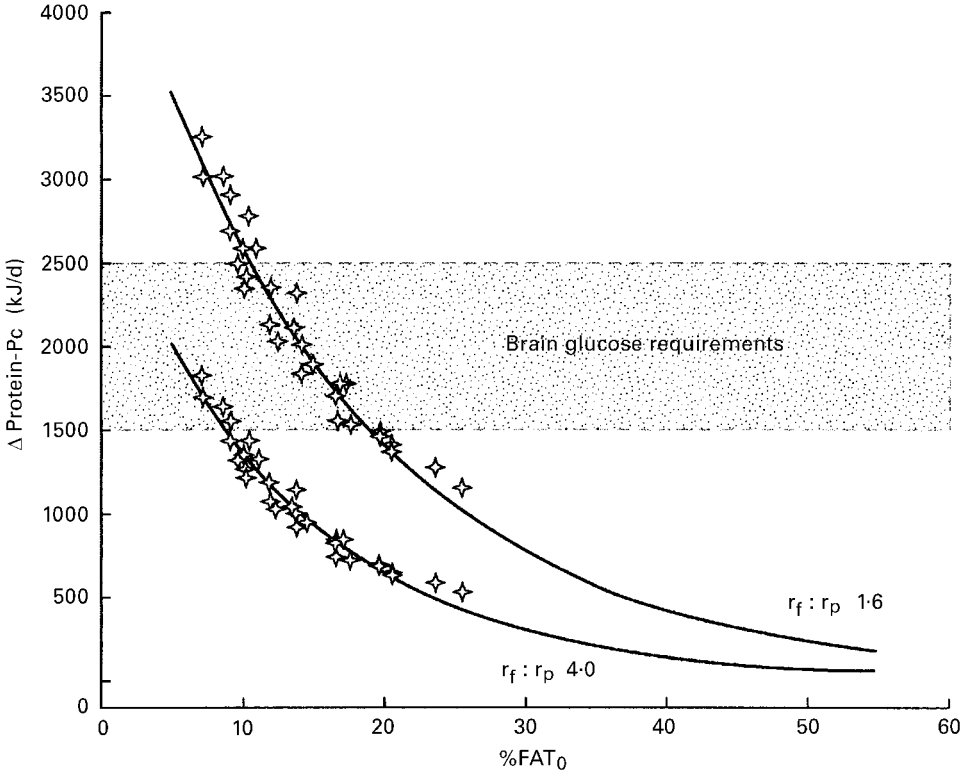

Fig. 9. Model prediction of the amount of energy that can be derived from protein mobilization entirely on the basis of the theoretical partitioning characteristic (Pc) of the individual (Δprotein-Pc) plotted as a function of the initial percentage body fat (%FAT_0). The calculations are based on data of BMR and percentage body fat of the Minnesota men (Keys *et al.* 1950) during the control (prestarvation) period, and the Δprotein-Pc is calculated as follows. Step 1: using equation 10 of the model, a minimum Pc and a maximum Pc of each of the Minnesota men can be calculated from his initial percentage fat, and with the values of fat-reserve fraction : protein-reserve fraction ($r_f : r_p$) between the limits shown in Fig. 8, i.e. with a minimum $r_f : r_p$ value of 1·6 and a maximum $r_f : r_p$ value of 4·0, i.e. 2SD below and above the mean $r_f : r_p$ value obtained from Fig. 7. Step 2: since Pc is the fraction of energy mobilized from protein, and since during total starvation (zero energy intake) the total energy mobilized under sedentary conditions is close to the BMR before starvation (BMR_0), the amount of energy mobilized from protein due to Pc (referred to as Δprotein-Pc) for each of the Minnesota men can be described as: Δprotein-Pc (kJ/d) = Pc × BMR_0. Step 3: these values for Δprotein-Pc, obtained with a minimum Pc and a maximum Pc are plotted against the initial percentage fat (with an exponential fit across the data), and compared with the energy requirements for glucose-requiring tissues or organs in the absence of any adaptation in fuel requirements. This is taken as 100 g glucose/d (Sokoloff, 1960) which, taking into account the fact that some glucose is provided by glycerol (released as a result of lipolysis in adipose tissue), would correspond to the mobilization of 150 g protein/d (Frayn, 1996), i.e. approx. 2500 kJ/d. Since this threshold of about 2500 kJ/d would be lower if the overall energy requirements of the glucose-requiring tissues falls in response to starvation (unproven but not impossible), an arbitrary lower limit (about 1500 kJ/d) has also been placed in the Fig.

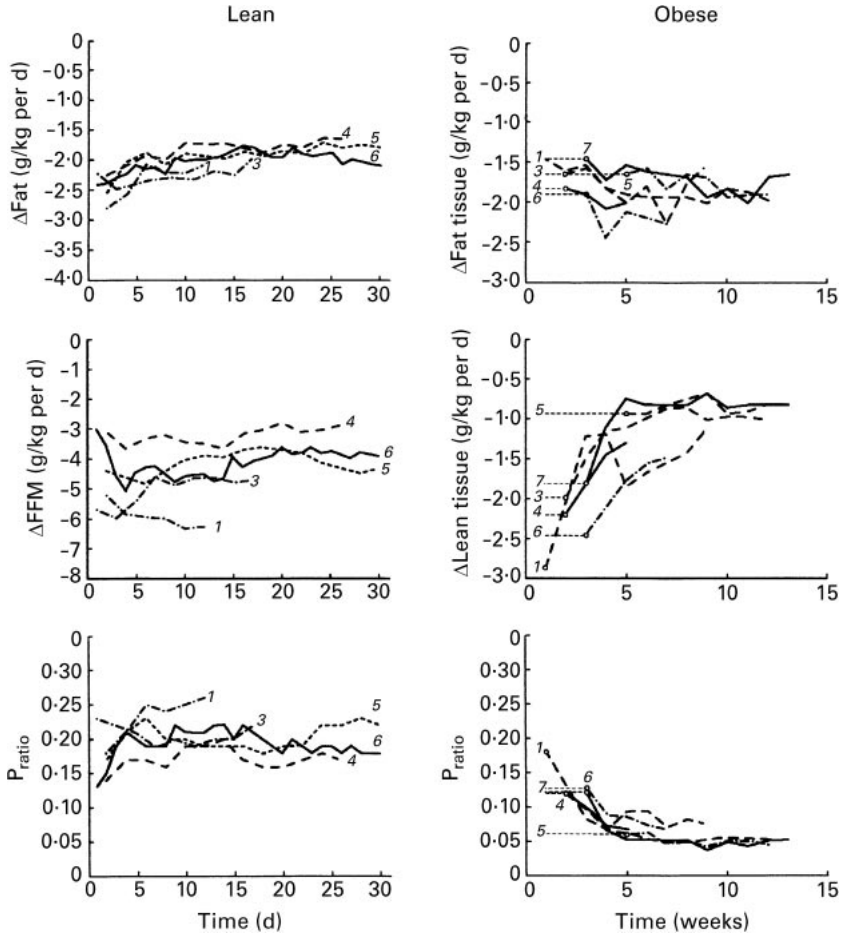

Fig. 10. Temporal changes in the fraction of energy mobilized as protein (P_{ratio}) and in body composition during the course of prolonged fasting, contrasting the relatively stable P_{ratio} in lean individuals with the fall in the P_{ratio} in the obese patients (over several weeks) before reaching relative stability. For lean individuals, the results are derived from data provided by Benedict (1915) and by Takahira (1925) who made sequential measurements of body weight, urinary nitrogen loss and BMR in individuals fasted for periods ranging between 12 and 31 d. Since no food was eaten and physical activity was minimal, the ratio urinary nitrogen losses : BMR is taken as a proxy for the fraction of energy lost as protein, i.e. the P_{ratio}, and is calculated according to the following equation:

$$P_{ratio} = \frac{\text{urinary N loss (g)} \times 6{\cdot}25 \times 18{\cdot}6}{\text{BMR}},$$

where 6·25 is the conversion factor for nitrogen to protein, and 18·6 is the metabolizable energy equivalent for protein (Livesey & Elia, 1988). Similar calculations of fasting P_{ratio} from the data of Benedict (1915) and Takahira (1925), and the assumptions inherent in these calculations have previously been discussed by Henry (1984), Henry *et al.* (1988), Elia & Parkinson (1989), and Elia (1991). The data on changes in fat-free mass (FFM) are determined from the changes in protein losses (calculated from nitrogen losses), while those for body fat were calculated from the difference between body weight and FFM at various time-points during the progress of the fast. The data for one subject (no. 2) who developed fever in the Takahira (1925) fasting study were omitted in our analysis. For the obese individuals, the sequential changes in P_{ratio} and in body composition are determined from the work of Gilder *et al.* (1967*a*,*b*) which reported sequential changes in body fat and FFM during fasting in obese patients. The P_{ratio} is calculated according to equation 1 (see p. 341). Only patients who fasted continuously for more than 1 month are shown, and by this criterion, one patient (no. 2) whose period of continuous fasting never exceeded 2 weeks was omitted in our analysis. The broken (horizontal) lines correspond to the duration of pre-adaptation periods on a low-energy diet before the fast. Indeed, the design of this study (Gilder *et al.* 1967*a*,*b*) was such that the obese patients were put on a low-energy diet for periods varying between 0 and 4 weeks before the fast. It is observed that the longer this pre-fast period of adaptation (i.e. the longer the period for the brain to adapt to utilization of ketone bodies as fuel), the shorter the delay for their P_{ratio} to reach relative stability and hence the shorter the period of specific protein-sparing after the onset of the fast.

specifically mobilize protein would be required to supplement the deficit in protein requirements for gluconeogenesis (Marliss *et al*. 1971; Marliss, 1978), and their importance would diminish as ketone bodies became part of the fuel substrate for the brain, i.e. when the brain became less dependent on glucose for its fuel needs, and hence leading to a specific sparing of protein. At the other extreme, the model predicts that lean individuals (body fat $<12\,\%$), by virtue of their high Pc, have sufficient capacity to meet their brain's glucose requirements entirely via the protein derived from the partitioning mechanism underlying their high Pc (Fig. 9). In other words, lean individuals (particularly those with high r_p values) need not bring into action to any great extent the additional mechanisms that specifically mobilize protein for gluconeogenic needs, and they are hence unlikely to show any major protein-sparing effect.

These predictions can be shown to hold true, thanks to a handful of studies of prolonged fasting conducted in lean (Benedict, 1915; Takahira, 1925) and obese individuals (Gilder *et al*. 1967*a*,*b*) from which the data can be used to

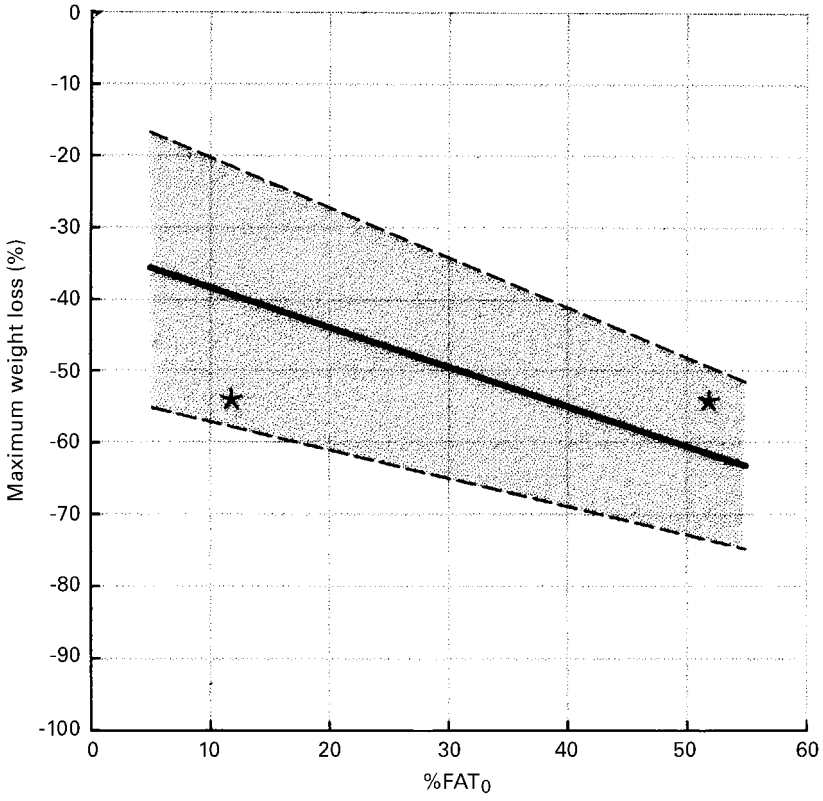

Fig. 11. Model prediction of maximum percentage weight loss as a function of the initial percentage body fat ($\%FAT_0$), using the equation describing maximum weight loss/initial weight ($\Delta W_R/W_0$) which is obtained as follows. Step 1: the maximum weight loss, ΔW_R, can be described as:

$$\Delta W_R = \Delta FFM_R + \Delta FAT_R,$$

where ΔFFM_R and ΔFAT_R are the changes in the protein-reserve and fat-reserve compartments, respectively. Step 2: using equations 6 and 7, ΔFFM_R and ΔFAT_R can be substituted so that ΔW_R can be rewritten as:

$$\Delta W_R = r_p \times FFM_0 + r_f \times FAT_0,$$

where r_p is the protein-reserve fraction, FFM_0 is the initial fat-free mass, r_f is the fat-reserve fraction and FAT_0 is the initial body fat. Step 3: using equations 3a and 3b, FFM_0 and FAT_0 in the equation above can be substituted to yield:

$$\frac{\Delta W_R\,(\%)}{W_0} = r_p \times \left(100 - \%FAT_0\right) + r_f \times \%FAT_0.$$

The middle line (—) is calculated with the mean value for r_p 0·33 and that for r_f 0·88, the upper line (---) with a maximum value for r_p (0·53) and for r_f (0·93), and the lower line (---) with a minimum value for r_p (0·13) and for r_f (0·83). The mean, minimum and maximum values for r_p and r_f are obtained from the mean ± 2SD values shown in the histograms in Fig. 7. The star symbols (★) help to illustrate the importance of r_p in determining the percentage weight loss; in particular that the maximum percentage weight loss in a typical lean individual with a high r_p can be similar to that of an obese individual with a low r_p.

calculate the temporal changes in P_{ratio} during the course of the fast (Gilder *et al*. 1967*a*,*b*; Henry *et al*. 1988; Elia, 1991). As shown in Fig. 10, the P_{ratio} of the lean individuals reaches relative stability within only 4–7 d after the onset of the fast, whereas the P_{ratio} in obese individuals, although initially at the level found in the lean, decreases slowly to reach relative stability only several weeks later. Their slow reduction in P_{ratio} and much longer delay in reaching the zone of relative stability of their P_{ratio} is clearly due to a specific sparing of protein since, as shown in Fig. 10, it results from a reduction in the rate of lean tissue loss whereas the rate of fat loss is practically unchanged. In other words, the decrease in P_{ratio} (protein-sparing effect) in the obese during the first few weeks of fasting is not due to a shift in the Pc of the individual, but to the subsequent 'downregulation' of mechanisms that initially brought about the specific increase in protein mobilization (in excess of that determined by their low Pc) during the time-period necessary for ketone bodies to become part of the fuel substrate for the brain. In the lean, by contrast, the rapid entry into the zone of stability of their P_{ratio} and hence the lack of specific protein-sparing effect during the course of starvation, implies that they meet their brain's glucose requirements almost entirely via their high Pc. Coupled with the findings that $\Delta FAT : \Delta FFM$ is greater than $FAT_0 : FFM_0$ during starvation (Fig. 4), the rapid entry into the zone of stability of the P_{ratio} and hence the lack of specific protein-sparing effect during the course of starvation in the lean also implies that (a) the effectiveness of protein sparing is set at the beginning of the starvation (Le Maho *et al*. 1988), and that (b) the well-known reduction in the rate of urinary N loss that continues to occur during the progress of starvation is not due to a specific sparing of the body's protein, but due to a general reduction in metabolic rate (Henry *et al*. 1988).

From this analysis showing differential temporal changes in P_{ratio} in lean and obese individuals undergoing prolonged fasting, it can be concluded that it is the Pc of the individual that determines the extent to which additional mechanisms specific for protein mobilization are recruited, the extent to which ketone bodies become fuel substrate for the brain, and hence the extent of the protein-sparing effect. Although the model predictions in Fig. 9 suggest that lean individuals with body fat $<12\,\%$ are likely to meet their brain energy requirements entirely on the basis of their Pc irrespective of their r_p, and obese individuals with body fat $>30\,\%$ would be clearly deficit *vis-à-vis* brain energy requirements irrespective of their r_p, they also suggest that it is in individuals with body fat of intermediate values (12–30 %) that variability in r_p is of critical importance in determining the brain's energy requirements entirely on the basis of the Pc of the individual, and hence the extent of subsequent protein-sparing.

V. Partitioning characteristic and variability in maximum percentage weight loss from starvation

The model can also be used to appreciate the quantitative importance of variations in the two determinants of Pc (initial percentage fat and r_p) in determining the maximum percentage weight loss from starvation. According to the model prediction shown in the plot in Fig. 11, the percentage weight loss in a typical lean individual with a high r_p can be similar to that of an obese person with a low r_p. The predictions of the model are not only in line with human data (Mitchell & Truswell, 1987; Elia, 1991) indicating that the mean values for maximum weight loss are 35–40 % for lean and 50–55 % for obese individuals, but they are also consistent with the large variability in maximum percentage weight loss for a given initial percentage fat on the basis of variability in r_p. Among those who could be classified under an initial 'lean' body weight are the thirty Irish hunger strikers who after 70 d total starvation (and sadly ten deaths) had a mean weight loss of 40 %, with individual values ranging between 30 and 50 % (Allison, 1992). Furthermore, losses of body weight exceeding 50 % in terminal cases of hunger-disease were observed by Jewish physicians of the Warsaw ghetto (Fliederbaum, 1979), and patients with anorexia nervosa losing up to 60 % of body weight have also been reported (Berkman *et al*. 1947). Among the obese, on the other hand, several cases of massive successful weight reduction beyond 60 % weight loss and up to 75 % have been reported during fasting in grossly obese patients (Bortz *et al*. 1967; McFarlan, 1991), and documented by Elia (1991).

Concluding remarks and perspectives

The focus in the present paper that the Pc of the individual during starvation is determined not only by the pre-starvation ratio fat : protein compartments ($\%FAT_0$), but also by the ratio of the two energy-reserve fractions ($r_f : r_p$), coupled with our previous demonstration from the Minnesota data that the Pc of the individual during starvation is conserved during refeeding (Dulloo *et al*. 1996), suggests that the initial percentage fat and the $r_f : r_p$ together provide a 'memory of partitioning' which defines the Pc of the individual, and determines not only the pattern of protein and fat mobilization during energy deficit, but also the way energy deposited during refeeding is partitioned in the protein and fat compartments. From the standpoint of system physiology, the 'memory of partitioning' dictates an autoregulatory control system that underlies the partitioning between protein and fat during weight loss and weight recovery, and its functional significance would be two-fold:

(a) during starvation: to meet the fuel needs of the individual in such a way that the energy-reserve component in both the protein and fat compartments would reach complete depletion simultaneously, a strategy that would ensure maximum duration of survival during long-term food scarcity, and
(b) during refeeding: to restore the two energy-reserve fractions in the protein and fat compartment in the same proportion as before starvation, and hence to re-establish the individual's pre-starvation capacity for survival during long-term food scarcity.

In addition to the autoregulatory control of partitioning *per se*, other control systems operating via the control of food intake and thermogenesis with feedback loops from the fat and/or lean tissue compartment have also been proposed in a conceptual model of autoregulation of body

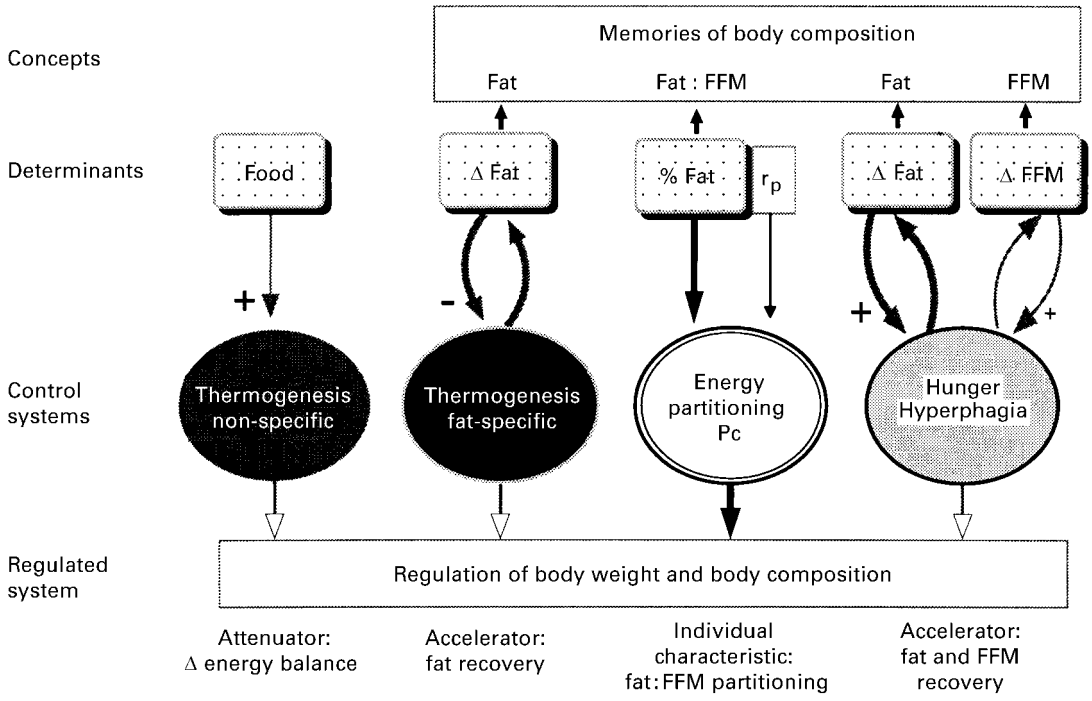

Fig. 12. A conceptual model for autoregulation of body composition during weight recovery from starvation (adapted from Dulloo, 1997). Pc, partitioning characteristic of the individual; r_p, fraction of the protein compartment which can function as an energy reserve; FFM, fat-free mass; Δ, change. The components of this model are derived from a series of papers (including the present one) based on re-analysis of data from the Minnesota experiment (Keys *et al.* 1950), and centre on distinct autoregulatory control systems outlined here. (1) The control of energy partitioning between protein and fat confers to the individual his or her Pc during starvation and subsequent weight recovery (Dulloo *et al.* 1996). It is dictated by a memory of the initial body composition, i.e. by the initial ratio fat : FFM (i.e. initial percentage fat) and by r_p. (2) Thermogenesis, which is suppressed during weight loss, remains suppressed during weight recovery by a magnitude which was proportional to the state of depletion of the fat stores, but unrelated to state of FFM depletion (Dulloo *et al.* 1996; Dulloo & Jacquet, 1998). This has led to the concept for the existence of a memory of the largest fat stores reached in a given individual, i.e. a 'fat-stores memory' which governs the suppression of thermogenesis as a function of the replenishment of the fat stores. The functional importance of the economy in thermogenesis during weight recovery is therefore to accelerate specifically the replenishment of the fat stores, thereby contributing to the disproportionate rate of fat relative to lean tissue recovery. This control of 'fat-specific' thermogenesis functions as a specific accelerator of body fat during weight recovery. It is distinct from the control of 'non-specific' thermogenesis which functions as an attenuator of energy imbalance, and is dictated by the food energy flux rather than by fat depletion–repletion. (3) Hunger–appetite drive leads to hyperphagia during weight recovery, and the magnitude of this hyperphagic response is determined by the extent to which body fat and FFM are depleted, with the degree of fat depletion being the stronger determinant (Dulloo *et al.* 1997). The hyperphagic response therefore seems to be dictated not only by a memory of the initial fat stores but also by a memory of the initial FFM (hence lean tissue) compartment. The functional importance of this increase in the hunger–appetite sensation, with consequential hyperphagia, is to accelerate the restoration of both lean and fat compartments, as defined by the Pc of the individual.

composition based on re-analysis of data from the Minnesota experiment (Dulloo, 1997; Dulloo *et al*. 1997; Dulloo & Jacquet, 1998), and they are conceptualized to be dictated by 'memories' of the fat and/or FFM compartment. As shown in Fig. 12, they are however viewed as 'attenuators' of energy imbalance and/or 'accelerators' of tissue recovery that are superimposed over a more 'basal' control of energy-partitioning which is dictated by the 'memory' of partitioning.

On the basis of the findings here that variability in Pc among the Minnesota men is primarily determined only by the variability in the initial percentage fat and in r_p (see first application of the model), it follows that the 'memory of partitioning' during starvation–refeeding is determined essentially by the ratio fat : protein tissue compartment before starvation (reflected in the initial percentage fat) and by the size of the energy-reserve fraction of the protein compartment (r_p). Future studies would need to elucidate the metabolic basis of this 'memory of partitioning' and to explore whether inter-individual variability in r_p resides in the ratio visceral mass : skeletal mass, in the fibre and/or biochemical composition of the skeletal muscle mass or in variability in skeletal muscle metabolism. The elucidation of the factors underlying variations in r_p would have important clinical implications *vis-à-vis* the pathophysiology of starvation since r_p is likely to be a critical global variable underlying differential susceptibilities to various functional impairments in response to protein–energy malnutrition or to therapeutic weight reduction, as well as to variability in the recovery of bodily functions during refeeding after cachexia. Furthermore, alterations in r_p might also be implicated in the loss of lean tissue mass that occurs during the 'normal' ageing process and in the greater susceptibility of the elderly to functional impairment during weight loss. Finally, the mathematical model described in the present paper (equation 10) could be useful for calculating the r_p from a knowledge of the initial percentage fat, Pc and r_f of the individual. Since the initial

percentage fat and body weight can be measured, and r_f can be estimated (using equation 11), the future challenge in elucidating the physiological basis of human variability in fuel partitioning resides in devising appropriate methodological approaches (less drastic than prolonged starvation) to measure the Pc of the individual.

References

Allison SP (1992) The uses and limitations of nutritional support. *Clincial Nutrition* **11**, 319–330.

Armitage P & Berry G (1987) *Statistical Methods in Medical Research*, 2nd ed. Oxford: Blackwell Scientific Publications.

Ashley BCE & Whyte HM (1961) Metabolic studies in starvation. *Australian Annals of Medicine* **10**, 92–111.

Benedict FG (1915) *A Study of Prolonged Fasting*. *Carnegie Institute of Washington Publication* no. 203, Washington, DC: Carnegie Institute.

Berkman JM, Weir JF & Kepler EJ (1947) Clinical observations on oedema, serum protein and the effects of forced feeding in anorexia nervosa. *Gastroenterology* **9**, 357–390.

Bortz WM, Wroldson A & Morris P (1967) Fat, carbohydrate, salt and weight loss. *American Journal of Clinical Nutrition* **20**, 1104–1114.

Cahill GF (1970) Starvation in man. *New England Journal of Medicine* **282**, 668–675.

Dulloo AG (1997) Human pattern of food intake and fuel-partitioning during weight recovery after starvation: a theory of autoregulation of body composition. *Proceedings of the Nutrition Society* **56**, 25–40.

Dulloo AG (1998) Partitioning between protein and fat during starvation and refeeding: is the assumption of intra-individual constancy of P-ratio valid? *British Journal of Nutrition* **79**, 107–109.

Dulloo AG & Jacquet J (1998) Adaptive reduction in basal metabolic rate in response to food deprivation in humans: a role for feedback signal from the fat stores. *American Journal of Clinical Nutrition* **68**, 599–606.

Dulloo AG, Jacquet J & Girardier L (1996) Autoregulation of body composition during weight recovery in humans: the Minnesota Experiment revisited. *International Journal of Obesity* **20**, 393–405.

Dulloo AG, Jacquet J & Girardier L (1997) Poststarvation hyperphagia and body fat overshooting in humans: a role for feedback signals from lean and fat tissues. *American Journal of Clinical Nutrition* **65**, 717–723.

Elia M (1991) Effect of starvation and very low calorie diets on protein–energy interrelationships in lean and obese subjects. In *Protein–Energy Interactions*, pp. 249–284 [NS Scrimshaw and B Schurch, editors]. Lausanne, Switzerland: IDECG.

Elia M & Parkinson S (1989) Protein economy during human starvation. *European Journal of Clinical Nutrition* **43**, 139–142.

Ferro-Luzzi A, Branca F & Pastore G (1994) Body mass index defines the risk of seasonal energy stress in the Third World. *European Journal of Clinical Nutrition* **48**, Suppl. 3, S165–S178.

Fliederbaum J (1979) Metabolic changes in hunger disease. In *Hunger Disease: Studies by Jewish Physicians in the Warsaw Ghetto*. New York, NY: John Wiley & Sons.

Forbes GB (1987) Lean body mass–body fat interrelationship in humans. *Nutrition Reviews* **45**, 225–231.

Forbes RM, Cooper AR & Mitchell HH (1953) The composition of the human body as determined by chemical analysis. *Journal of Biological Chemistry* **203**, 359–366.

Forbes RM, Mitchell HH & Cooper AR (1956) Further studies on the gross composition and mineral elements of the adult human body. *Journal of Biological Chemistry* **223**, 969–975.

Frayn KN (1996) *Metabolic Regulation: A Human Perspective*, pp. 166–174. London: Portland Press Ltd.

Gilder H, Cornell GN, Grafe WR, Macfarlane FR, Asaph JW, Stubenbord WT, Watkins GM, Rees JR & Thorbjarnarson B (1967*a*) Components of weight loss in obese patients subjected to prolonged starvation. *Journal of Applied Physiology* **23**, 304–310.

Gilder H, Cornell GN & Thorbjarnarson B (1967*b*) Human energy expenditure in starvation estimated by expired air analysis. *Journal of Applied Physiology* **23**, 297–303.

Grande F (1964) Man under caloric deficiency. In *Handbook of Physiology. Adaptation to the Environment*, vol. 4, pp. 911–937. Washington, DC: American Physiology Society.

Heitmann BL (1990) Evaluation of body fat estimated from body mass index, skinfolds and impedence. A comparative study. *European Journal of Clinical Nutrition* **44**, 831–837.

Henry CJK (1984) Protein : energy inter-relationships and the regulation of body composition. PhD Thesis, University of London.

Henry CJK, Ghusain-Choueiri A & Payne RR (1998) Energy partitioning between body storage of protein and fat during starvation and refeeding: sources of intra-individual variation of P-ratios. *British Journal of Nutrition* **79**, 109–110.

Henry CJK, Rivers J & Payne RR (1988) Protein and energy metabolism in starvation reconsidered. *European Journal of Clinical Nutrition* **42**, 543–549.

Keys A, Brozek J, Henschel A, Mickelson O & Taylor HL (1950) *The Biology of Human Starvation*. Minneapolis, MN: University of Minnesota Press.

Leiter A & Marliss EB (1982) Survival during fasting may depend on fat as well as on protein stores. *Journal of the American Medical Association* **248**, 2306–2307.

Le Maho Y, Robin JP & Cherel Y (1988) Starvation as a treatment for obesity: the need to conserve body protein. *News in Physiological Science* **3**, 21–24.

Livesey G & Elia M (1988) Estimation of energy expenditure, carbohydrate utilization, and net fat utilization and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. *American Journal of Clinical Nutrition* **47**, 608–628.

Marliss EB (1978) The physiology of fasting and semistarvation: roles of 'caloristat' and 'aminostat' mechanisms. In *Recent Advances in Obesity Research*, vol. 2, pp. 345–369 [GA Bray, editor]. Westport, CT: Food and Nutrition Press.

Marliss EB, Aoki TT, Unger RH, Soeldner JS & Cahill GF (1971) Glucagon levels and metabolic effects in fasting man. *Journal of Clinical Investigation* **49**, 2256–2270.

McFarlan D (1991) *The Guinness Book of Records*. Barcelona, Spain: Guinness Publishing Co.

Meyers AW (1917) Some morphological effects of prolonged initiation. *Journal of Medical Research* **36**, 51–77.

Millward DJ & Wijesinghe DGNG (1998) Energy partitioning and the regulation of body weight. *British Journal of Nutrition* **79**, 111–113.

Mitchell PB & Truswell AS (1987) Body composition in anorexia nervosa and starvation. In *Handbook of Eating Disorders*, part 1: *Anorexia and Bulimia Nervosa*, pp. 45–77 [PJV Beumont, GD Burrows and RC Casper, editors]. Amsterdam: Elsevier.

Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG & Cahill GF (1967) Brain metabolism during fasting. *Journal of Clinical Investigation* **46**, 1589–1595.

Passmore R, Strong JA & Ritchie FH (1958) The chemical composition of the tissue lost by obese patients on a reducing regimen. *British Journal of Nutrition* **12**, 113–122.

Payne PR & Dugdale AE (1977) Mechanisms for the control of body weight. *Lancet* **i**, 583–586.

Prentice AM, Goldberg GR, Jebb SA, Black AE, Murgatroyd PR & Diaz EO (1991) Physiological responses to slimming. *Proceedings of the Nutrition Society* **50**, 441–458.

Sokoloff L (1960) Metabolism of the central nervous system *in vivo*. In *Handbook of Physiology*. Section I, *Neurophysiology*, pp. 1843–1864. Baltimore, MD: Waverly Press Inc.

Strong JA, Passmore R & Ritchie FJ (1958) Clinical observations on obese patients during a strict reducing regimen. *British Journal of Nutrition* **12**, 105–112.

Takahira H (1925) Metabolism during fasting and subsequent refeeding. *Imperial Government Institute for Nutrition (Tokyo)* **1**, 63–82.

Voit E (1901) Die Grösse des Eiweisszerfalles im Hunger (Extent of protein catabolism during starvation). *Zeitschrift für Biologie* **16**, 167–195.